

VISIT...

LANZAROTE
Caliente.COM

12

Flowing On: The Future

In the Preface, I referred to the way flow cytometry has moved over the past three decades in directions that have surprised even those intimately involved in the field. It is obvious that attempts to predict the future of flow cytometry should be left to informal discussions among friends. The temptation to speculate is, however, an impossible one to resist. This chapter should therefore be read in the spirit in which it was written: with a large measure of skepticism (preferably among friends, after a good dinner and a good bottle of wine).

The capabilities of state-of-the-art research cytometers should, over the next years, continue to move toward easier analysis of particles of greater size range with more sensitivity and greater speed than they do now. Although currently including particles from over 100 μm (like plant protoplasts) down to bacteria and DNA fragments on research instruments, this range may soon become possible on benchtop cytometers. Exploitation of this flexibility may finally lead to greater use of flow cytometers for looking at plant cells and also at microbiological systems. It may also be possible to use cytometers to look at the properties of isolated organelles such as mitochondria and chloroplasts. It is likely that some type of flow cytometry will begin to be used more routinely in bacteriology and environmental laboratories. Recent developments with multiplexed bead-based assays lead many to guess that flow cytometry is also poised to enter the biochemistry laboratory, as an alternative to the ELISA technique for assaying soluble components.

High-speed drop sorters may continue to increase in speed, although the high pressures required in these sorters may have reached their limit in terms of the viability of the sorted cells. Optical sorting (by using a laser to inactivate unwanted cells or chromosomes) may

begin to provide a practical, rapid alternative to drop sorting. An obvious method for allowing more effective use of time and sample on conventional drop sorters would be to sort four or more populations (instead of just two) simultaneously. Although slow to catch on, four-population sorting is now available on commercial instruments. This involves charging some drops more than others so that drops can be deflected slightly to the left and to the right as well as strongly to the left and to the right—giving four options and allowing four populations to be sorted during each run. Four populations is not the technical limit—if biologists can think of experiments that demand more.

Extra lasers have been appearing even now on benchtop cytometers; excitation lines may continue to become less limiting in the choice of fluorochromes and the design of experiments. Developments in fluorochrome technology, in conjunction with extra lasers, have already facilitated super-multiparameter analysis by allowing the use of wider regions of the spectrum without unsurmountable problems of spectral overlap. Whereas routine instruments today look at forward and side scatter as well as three or four fluorescence parameters, some high-tech instruments already quite happily look at 11 fluorescence parameters (plus forward and side scatter), and, with demand, the electronics are capable of handling even more. The question really concerns, then, the ability of the human intellect to plan rational experiments and to cope with the information that can be obtained in a short period of time from particles that are analyzed for large numbers of parameters. Even after more than a decade of multiparameter benchtop cytometry, it is not clear that our ability to design meaningful experiments with multiple measured parameters has yet caught up with our ability to measure those parameters. Despite the ability to look simultaneously at three or four fluorescent stains quite routinely on many instruments, most people still seem quite happy to stick to two. Whether this is related to general conservatism, the high cost of additional antibodies, problems with cross-reactivity in complex staining systems, or some inherent human discomfort with forays into multidimensional reasoning is a question that may be answered in the future.

There may well be increasing interest in analysis of the intrinsic characteristics of cells that lead to alterations in autofluorescence. John Steinkamp, Harry Crissman, and others at the Los Alamos Laboratory have been using flow systems to study the time character-

istics (that is, the decay kinetics) of fluorescence emission, as a handle for studying alterations in the microenvironment of fluorochromes bound to molecules in the cell; their results are exciting and might be used to study autofluorescence in the future. On a larger time scale, biochemical kinetics experiments may become more popular with increasing use of functional probes. It may also happen that flow cytometrists and microscopists will begin, at last, to talk to each other when they discover that they share common interests in image analysis microscopy. Laser-scanning cytometry already marks the beginning of methodological communication between the analysis strategies typical of flow cytometry and the microscope-based comfort zone required by pathologists. A cytometry laboratory of the future may well be equipped with a collection of fluorescence and confocal microscopes, laser-scanning cytometers, and flow cytometers—all being used, as appropriate, by the same people.

The extra information collected by rapid cytometers looking at many parameters will certainly increase the demand on data storage systems. Ten years ago, when writing the first edition of this book, I said that, against general scientific practice, flow cytometrists might need to begin to learn to wipe out data that they no longer need. Less expensive media for data storage have now made it possible to avoid such measures. Zip cartridges and CD-ROMs are the media of choice now; DVDs may be the choice in the near future (and the next “perfect” storage medium may already be on someone’s drawing board). Given the easier availability of inexpensive storage media, I see no reason to expect that our demands for data storage capacity will do anything other than continue to increase. With regard to software, the trend may continue toward a healthy proliferation of varied packages for flow analysis with full compatibility of data acquired on any and all systems.

I said at the beginning of this book that flow cytometry is currently moving in two directions at once: Technological advances provide, in one direction, increasingly rapid, sensitive, complex, but precarious analysis and, in the other direction, increasingly stable, fool-proof, and automated capabilities. Thirty years ago, all flow cytometry was at the complex, but precarious level of development. Now, many of those early precarious developments have been incorporated into routine cytometers, and new techniques are appearing in research instruments. Over the next few years, this progression will continue:

The vast majority of future flow cytometric analysis will be done using routine, black-box instruments of increasingly greater capabilities. In association with this trend, expert (artificial intelligence) systems may begin to have an impact. Robotic systems, incorporating staining, lysing, and centrifugation steps, may allow staff to put blood or other crude material in at one end and get formatted print-outs of flow data from the other. A remaining question is whether fluidics stability, reagent quality control, and subjectivity in gating will ever allow us to relax with this level of automation.

And what does the future hold for the *spirit* of flow cytometry? Although there will continue to be many new technical developments (some predicted and some surprising), I suspect that the sense of adventure may be lost from the field as the balance continues to shift from innovative technology to routine use. The trade-off for that loss of excitement could be the satisfaction that people who understand flow cytometry may feel from having played a part in the comfortable acceptance of this powerful technique by the broad scientific community.

FURTHER READING

Cualing HD (2000). Automated analysis in flow cytometry. *Cytometry* 42:110–113.

Durack G, Robinson JP, eds. (2000). *Emerging Tools for Single Cell Analysis: Advances in Optical Measurement Technologies*. Wiley-Liss, Inc, New York.

Roslaniec MC, Bell-Prince CS, Crissman HA, et al. (1997). New flow cytometric technologies for the 21st century. *Hum. Cell* 10:3–10.